NOTE TO FILE

Subject: Monsanto's Glyphosate Tolerant Canola Line GT73

Keywords:
Canola, *Brassica napus*, Glyphosate Tolerant, Herbicide Tolerant, CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS), Glyphosate oxidoreductase (GOX), Roundup™ Ready.

Background

On March 6. 1995, at Monsanto's request, a meeting was held to discuss their approach to evaluating the safe food use of canola line GT73. In a submission dated March 31, 1995, Monsanto provided summary information to support their safety assessment of canola line GT73.

Molecular Alterations and Characterization of Canola Line GT73

Based on polymerase chain reaction (PCR) and restriction fragment analyses of total DNA isolated from transgenic canola line GT73 and the parental Westar line, Monsanto has concluded that their canola line GT73 is the result of one insertion event.

Using PCR analysis, Monsanto reported that they have mapped the left and right borders of the transgenic DNA and concluded that canola line GT73 does not contain any DNA derived from sequences outside of the left and right borders of the binary vector used to develop canola line GT73. At the insertion site, the transgenic DNA is composed of DNA sequences that direct the expression and cellular targeting of the CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) and glyphosate oxidoreductase (GOXv247) proteins.

Monsanto reports that the CP4-EPSPS gene was isolated from *Agrobacterium* sp. strain CP4. Monsanto engineered the CP4-EPSPS gene to contain the chloroplast targeting sequences from the *Arabidopsis thaliana* EPSPS gene (AEPSPS/CTP2). Monsanto also reported that they optimized the CP4-EPSPS gene for plant expression.

According to Monsanto, the GOXv247 gene is a mutated version of the wild type GOX gene from *Achromobacter* sp. stain LBAA. The GOXv247 protein is >99% homologous to the wild type GOX from *Achromobacter* sp. strain LBAA. Monsanto engineered the GOXv247 gene to contain the chloroplast targeting sequences for the *Arabidopsis thaliana* small subunit 1A ribulose-1,5-bisphosphate carboxylase (Arab-SSU1A/CTP1). Monsanto also reported that they optimized the GOXv247 gene for plant expression.

Based on information provided by Monsanto, both the EPSPS and GOXv247 genes are each under the control of a 35S promoter from a modified figwort mosaic virus (P-CMOVb).

According to Monsanto, the inserted DNA is capable of expressing two new proteins: 1) CP4-EPSPS and 2) GOXv247. Based on the PCR analysis, genomic restriction fragment mapping, and genetic analysis, Monsanto has concluded that canola GT73 line contains one intact copy of both the *CP4-EPSPS* and *GOXv247* transgenes and they are integrated at one site in the nuclear genome. This conclusion is supported, in part, by genetic analyses demonstrating that the glyphosate tolerance DNA segregates as a single Mendelian genetic trait. Moreover, based on genomic DNA restriction analysis, Monsanto reports that the restriction pattern of the transgenic DNA is stably inherited in all generations they have examined.

Expressed Protein

As stated above, the only new proteins expected to be expressed in canola line GT73 are CP4-EPSPS and GOXv247. Monsanto examined the heat stability and pH optima of CP4-EPSPS and GOX. These proteins are not expected to be a component of canola oil, but are expected to be present in canola meal.

According to Monsanto, the pH optima for CP4-EPSPS and GOX activity is pH 9.0 - 9.5 and pH 6.5 - 7.0, respectively. No CP4-EPSPS activity was measured at pH values less than pH 5. Monsanto reports that at very low pH in simulated gastric fluids, GOX activity is eliminated in 0.25 minutes.

According to information provided by Monsanto, upon incubation at 55 °C for 15 minutes, more than 50% of the CP4-EPSPS activity observed at 25 °C is lost and all CP4-EPSPS activity is lost after incubation for 15 minutes at 65 °C. Monsanto reports that upon incubation at 60 °C for 15 minutes, 83% of the GOX activity measured at 25 °C is lost. Monsanto reports, that for comparison, canola flakes are heated approximately 30 to 40 minutes at 103 to 107 °C during the routine preparation of canola meal for animal feed.

Compositional Analysis

Based on the nature of the genetic modification, it is expected that canola line GT73 would not differ materially in composition from other canola varieties. To confirm this expectation, Monsanto analyzed the composition of canola seed, canola oil, and canola meal obtained from line GT73 and a Westar control line. According to Monsanto, canola oil is a commonly used oil for human consumption. Heat-processed canola meal is commonly used as a protein supplement in animal feed (livestock, poultry, pet food, fish food) and is considered a good source of the sulfur containing amino acids methionine and cystine.

Monsanto has concluded that canola seeds from canola line GT73 are not materially different from canola seeds from the parental Westar variety. Monsanto provided a compositional analysis of canola seeds from GT73 and Westar. They reported that there were no meaningful differences in the level of protein, oil, fiber, ash, and gross energy. In addition, they reported no meaningful difference between seeds derived from canola lines GT73 and Westar in the levels of amino acids, including the aromatic amino acids phenylalanine, tyrosine, and tryptophan both on a per protein and a per seed basis. Moreover, they determined at the 95% confidence level that the alkyl glucosinolates and erucic acid levels in canola seed from line GT73 will not exceed the established limits of 30 μmoles/g defatted meal and 2% of the oil composition, respectively. Monsanto also analyzed the level of sinapine in canola seed and could detect no difference in the sinapine level in seeds derived from GT73 and Westar varieties.

Based on their analyses, Monsanto has concluded that there is no meaningful difference between canola oil derived from canola line GT73 and canola oil derived from other canola varieties. To support their conclusion, Monsanto compared the fatty acid composition of oil derived from GT73 and Westar canola varieties. Monsanto reported no meaningful difference between the fatty acid composition (C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, C20:2, C22:0, or C22:1) of oil derived from GT73 and Westar canola varieties. Monsanto also measured the erucic acid level in canola oil derived from GT73 and concluded that there is 95% confidence that the level will not exceed the the 2% maximum limit.

Monsanto has also concluded that there is no meaninful difference between canola meal derived from their canola line GT73 and other canola varieties in their content of: protein, fiber, fat, ash, moisture, carbohydrate, energy, and in the levels of glucosinolates, amino acids, mineral composition, phytic acid content, and nitrogen solubility.

Wholesomeness Studies

Monsanto reported their results from animal feeding studies designed to determine the wholesomeness and safety of glyphosate tolerant canola. From two 4-week rat feeding studies with unprocessed and processed canola meal, Monsanto concluded that there was nothing in the study results that was unexpected based on the known effects of feeding canola meal to animals, and that the insertion of glyphosate tolerance genes into canola had no unintended effects on wholesomeness. From a 10-week trout feeding study with processed canola meal, Monsanto concluded that there were no adverse effects that were attributed to insertion of the glyphosate tolerance trait into canola. From two 5-day quail feeding studies with unprocessed canola meal, Monsanto concluded that there were no adverse effects observed that were attributed to insertion of the glyphosate tolerant traits into canola.

Conclusions

Monsanto has concluded that their canola line GT73: " . . . is not materially different from the parental canola line (Westar) and canola now being sold, in any meaningful way, except for the ability to tolerate glyphosate." Monsanto reported that: "[i]n all cases, data for GT73 were within established limits for canola ($<30\mu$mol glucosinolates/g defatted meal and $<2\%$ erucic acid), and ranges of literature values for nutrients." At this time, based on Monsanto's description of its data and analyses, the Agency considers Monsanto's consultation on their canola line GT73 to be complete.

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